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Timothée Fontolliet

Vincent Pichot

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TESTING THE VAGAL WITHDRAWAL HYPOTHESIS DURING LIGHT EXERCISE UNDER AUTONOMIC BLOCKADE: A HEART RATE VARIABILITY STUDY

Timothée Fontolliet[1], Vincent Pichot[2], Aurélien Bringard[1], Nazzareno Fagoni[3], Alessandra Adami[4], Enrico Tam[5], Raffaello Furlan[6], Jean-Claude Barthélémy[2], Guido Ferretti[1,3]

1. Départements d'Anesthésiologie, de Pharmacologie et des Soins Intensifs / des Neurosciences Fondamentales, Université de Genève, Geneva, Switzerland
2. EA SNA-Epis 4607, Université Jean-Monnet, Saint-Étienne, France
3. Dipartimento di Medicina Molecolare e Traslazionale, Università di Brescia, Brescia, Italy
4. Department of Kinesiology, College of Health Sciences, University of Rhode Island, RI, USA
5. Dipartimento di Scienze Neurologiche e del Movimento, Università di Verona, Verona, Italy
6. Division of Internal Medicine, Humanitas Clinical and Research Center, Rozzano Humanitas University, Rozzano (MI), Italy

Author's contributions

Fontolliet T: Experiment conception, data treatment, statistical analysis, preparation of the first draft of the manuscript.

Pichot V: Preparation of data treatment software, statistical analysis.

Bringard A: Experiment execution, rereading of the manuscript.

Fagoni N: Experiment execution.

Adami A: Experiment execution, rereading of the manuscript.

Tam E: Experiment execution.

Furlan R: Rereading of the manuscript with special references to heart rate variability technics.

Barthélémy JC: Rereading of the manuscript with special references to heart rate variability technics.

Ferretti G: Preparation of ethical committee folder, experiment conception, study design, patient selection, rereading of the manuscript.

Running head: Autonomic blockade and heart rate variability

Corresponding author:

Timothée Fontolliet

Départements d'Anesthésiologie, de Pharmacologie et des Soins Intensifs / de Neurosciences Fondamentales

Université de Genève

1, Rue Michel Servet

CH-1211 Genève 4, Switzerland

E-mail: timothee.fontolliet@unige.ch

Phone: 0041 22 3795365

Fax: 0041 22 3795402

ORCID: 0000-0002-4140-4234

ABSTRACT

Introduction. We performed the first analysis of heart rate variability (HRV) at rest and exercise under full autonomic blockade on the same subjects, to test the conjecture that vagal tone withdrawal occurs at exercise onset. We hypothesized that, between rest and exercise: i) no differences in total power (P_{TOT}) under parasympathetic blockade; ii) a P_{TOT} fall under β1-sympathetic blockade; iii) no differences in P_{TOT} under blockade of both ANS branches.

Methods. 7 males (24±3 years) performed 5-min cycling (80W) supine, preceded by 5-min rest during control and with administration of atropine, metoprolol and atropine+metoprolol (double blockade). Heart rate and arterial blood pressure were continuously recorded. HRV and blood pressure variability were determined by power spectral analysis, and baroreflex sensitivity (BRS) by the sequence method.

Results. At rest, P_{TOT} and the powers of low (LF) and high (HF) frequency components of HRV were dramatically decreased in atropine and double blockade compared to control and metoprolol, with no effects on LF/HF ratio and on the normalised LF (LFnu) and HF (HFnu). At exercise, patterns were the same as at rest. Comparing exercise to rest, P_{TOT} varied as hypothesized. For SAP and DAP, resting P_{TOT} was the same in all conditions. At exercise, in all conditions, P_{TOT} was lower than in control. BRS decreased under atropine and double blockade at rest, under control and metoprolol during exercise.

Conclusions. The results support the hypothesis that vagal suppression determined disappearance of HRV during exercise.

Key words

Cardiovascular regulation · Arterial blood pressure · Baroreflexes · Metoprolol · Atropine

New & Noteworthy

This study provides the first demonstration, by systematic analysis of heart rate variability (HRV) at rest and exercise under full autonomic blockade on the same subjects, that suppression of vagal activity is responsible of the disappearance of spontaneous HRV during exercise. This finding supports previous hypotheses on the role of vagal withdrawal in the control of the rapid cardiovascular response at exercise onset.

INTRODUCTION

At exercise start, the characteristics of the heart rate (HR) kinetics under vagal blockade (12) suggested that sudden withdrawal of vagal tone may occur. This hypothesis may explain the concomitant sudden increase of cardiac output (13, 25). Recently, vagal withdrawal was called upon also to explain the early changes in baroreflex sensitivity upon exercise start (4). If this is so, we should expect that the amplitude of the rapid HR and cardiac output responses would be greater, the stronger is the vagal modulation of heart activity at rest.

The experimental evidence, however, is not conclusive under this respect, and several data seem to contradict the vagal withdrawal hypothesis. For instance, although we know that resting vagal activation is greater in supine than in upright position (35, 47, 49), the amplitude of the rapid cardiac output response at exercise onset was found to be smaller in supine than in upright posture (27; 55). On the other hand, vagal activity is reduced and sympathetic activation is increased in acute hypoxia as compared to normoxia (5;18, 23, 57, 58): in spite of this, even in hypoxia HR determined a large fraction of a significant cardiac output response (26). These data represent a serious challenge to the vagal withdrawal hypothesis at exercise onset.

The vagal withdrawal hypothesis at exercise onset may also be tested by investigating the neural modulation of the heartbeat under pharmacological blockade of either the vagal or the sympathetic or both branches of the ANS (2, 6, 15, 17, 21, 24, 29, 32, 33, 35, 43, 53). The analysis of spontaneous heart rate variability (HRV) demonstrated that vagal blockade reduced the total power (P_{TOT}) of HRV, acting on the reduction of both its high (HF) and low frequency (LF) components. Nevertheless, little attention was given so far to the analysis of HRV during exercise combined with pharmacological blockade. Warren et al. (1997) reported that the powers of both the

LF and the HF peaks were by far lower at exercise than at rest under placebo, but they did not find differences under vagal blockade with glycopyrrolate; moreover, esmolol administration provided similar results as placebo. The interpretation of their results was undermined by the type of drug used and their study was limited by the fact that they did not analyse blood pressure variability, another important indirect feature of sympathetic modulation of the cardiovascular system. Polanczyk et al. (42) showed that atropine and propranolol administration did not vary the spectrum components of HRV, contrary to their expectations.

If the vagal withdrawal hypothesis was correct, we should predict that, when comparing rest and exercise: i) no differences in PTOT, LF and HF under full vagal blockade would be found; ii) a drastic fall in PTOT, LF and HF under selective β1-sympathetic blockade would occur; iii) no differences in PTOT, LF and HF under simultaneous blockade of the two branches of the ANS would appear. Moreover, we expected that arterial blood pressure variability would not follow the same pattern of response as HRV, because the former reflects more the peripheral sympathetic vascular modulation than the central cardiac modulation.

These predictions were tested in the present study, the aim of which was to investigate the effects of vagal blockade, of selective β1-sympathetic blockade and of simultaneous blockade of both branches of the ANS, at rest and during exercise, on HRV and blood pressure variability.

METHODS

Participants

Seven healthy non-smoking young participants volunteered for the experiments. They were (mean ± SD) 24.3 ± 2.6 years old, 181.2 ± 3.1 cm tall and weighed 78.9 ± 6.1 kg. Exclusion criteria were: presence of history of cardiopulmonary disease and regular use of drugs at the time of the study. Participants were instructed to avoid caffeine consumption 24 hours before the visit and to refrain from performing strenuous exercise the day before testing.

All participants were preliminarily informed on the design and risks associated with the experiments and they signed a written informed consent. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local institutional ethical committee.

Protocol and measurements

The experiments were carried out in the Clinical Physiology Laboratory of the University of Geneva, Switzerland. The volunteers reported to the laboratory on four different days, with at least a 48-hour recovery between visits. Experiments were performed in supine posture, in order to reduce potential mechanical effects related to the remarkable sudden increase in venous return at exercise start upright. After instrumentation, a 20-gauge catheter was placed in the antecubital vein of the right arm to administer drugs. A unique 5-min monitoring at rest preceded a series of three 5-min constant-load leg exercises, on cycle ergometer, at 80 watts, to avoid lactate threshold. Between repetitions a 5-min recovery was administered.

For the entire duration of the protocol, we obtained continuous recordings of the electrocardiogram (Elmed ETM 2000, Heiligenhaus, Germany), and the arterial pulse pressure profiles, obtained at a fingertip of the left arm by means of a non-invasive cuff pressure recorder (Portapres, FMS, Amsterdam, The Netherlands).

The R-R interval (RR) and its reciprocal, HR, were computed beat-by-beat. Systolic and diastolic blood pressure (SAP and DAP, respectively) values were

obtained from each pulse pressure profile, using the Beatscope® software package (FMS, Amsterdam, The Netherlands). Beat-by-beat mean arterial pressure (MAP) was computed as the integral mean of each pressure profile, using the same software package. Breathing frequency was also calculated from the electrocardiogram plot.

All the signals were digitalized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA) and stored on a computer. The acquisition rate was 400 Hz.

The protocol was performed under four experimental conditions, administered in random order: i) control, i.e. with placebo infusion, ii) parasympathetic blockade with atropine administration, ii) selective β1-adrenergic blockade with metoprolol administration, and iv) double blockade of both branches of the ANS with simultaneous atropine and metoprolol administration.

Drug administration

Parasympathetic blockade was achieved by administering atropine in a single 0.04 mg/kg dose (mean 3.06 ± 0.23 mg, range 2.7 – 3.4 mg), which was used in previous studies to attain full vagal blockade (14, 17, 31, 59). The half-life of a single atropine dose is 180 minutes (52) so that, blockade was maintained during the entire duration of each experiment.

The β1-adrenergic blockade was attained by using metoprolol tartrate (Loprésor, Novartis, Switzerland). After an initial bolus of 15 mg, metoprolol tartrate was continuously infused in an antecubital vein at a rate of 45 mg per hour, by means of an infusion pump. The efficacy of adrenergic blockade along time was evaluated on a separate session, by analysing the heart rate response following isoprenaline injection, as previously described (14). The correct metoprolol

maintenance dose was identified as the dose ensuring an 80% reduction of the HR response to isoprenaline for the entire protocol duration.

For the experiments with double, simultaneous sympathetic and parasympathetic blockade, the same atropine and metoprolol dose and administration procedure described here above were applied.

Data treatment

After construction of the time series of RR, SAP and DAP from the continuous recordings of electrocardiogram and pulse pressure profiles, Fast Fourier Transform (FFT) was used to evaluate spontaneous variability of RR, SAP and DAP (35). The data length used was 5 minutes at rest and 3 minutes at exercise. In the latter case, one repetition, that with the most stable and cleanest trace, was analysed. The total power (PTOT) (0.0-0.5 Hz) of RR, SAP and DAP variabilities, corresponding to variance, was initially computed. Subsequently, the powers and frequencies of LF (0.03–0.14 Hz) and HF (0.15–0.5 Hz) components of the power spectrum were computed and expressed in absolute units (ms^2). The very low frequency component was neglected. The LF/HF ratio was also calculated. Normalized LF and HF (LFnu and HFnu, respectively) were computed as:

$$\frac{\mathrm{LF}\times 100}{\mathrm{Ptot}-\mathrm{VLF}} \qquad (1)$$

and expressed in normalized units (28).

The spontaneous baroreflex sensitivity (BRS, expressed in ms $mmHg^{-1}$) was estimated from SAP and RR by means of the sequence method (3). Sequences of at least three heartbeats, corresponding to an increase or decrease in SAP and identifying a consensual change in RR interval, were selected. Linear regression analysis was applied on these sequences and the calculated slope was retained. BRS was then calculated as the mean of the slopes of all sequences per each

participant in each condition. Only sequences showing a coefficient of determination of at least 0.85 were analysed.

Spectral analysis and BRS were performed on Matlab® environment as previously described (41). Breathing Frequency was calculated with the ECG-Derived-Respiration method used by Moody et al. (30).

Statistics

Data are reported as group means ± standard deviation. The effects of medication and exercise type on the main outcomes were analysed by 2-way ANOVA for repeated measurements. When applicable, a Tukey post-hoc test was used to locate significant differences. Differences were considered significant if $p<0.05$. All data were analysed with Statistica 12 © (StatSoft, Inc., Tulsa, OK).

RESULTS

All participants successfully completed the study maintaining a normal sinus beat along the four experimental conditions (no arrhythmic beats were observed). The mean values of measured and calculated variables at rest and during exercise for all conditions are reported in Table 1. *At rest*, in control condition, HR was 62.7 ± 8.5 min^{-1}. Under sympathetic blockade, no significant differences with respect to control were observed. Under atropine, it was significantly higher than in control and under metoprolol. Under double blockade, it was higher than in control and under metoprolol, but lower than under atropine. *During exercise*, in control condition, HR was 105.0 ± 12.4 min^{-1}, and was higher under metoprolol, atropine and double blockade than in control. With respect to the corresponding values at rest, HR during exercise increased in all conditions except double blockade.

At rest, in control condition SAP was 112.0±9.5 mmHg and DAP was 55.0±9.6 mmHg. With respect to control, no differences were observed for either SAP or DAP with any investigated pharmacological treatment, although with double blockade, DAP tended to be higher than in control and was significantly higher than under metoprolol. MAP was 74.0±8.6 mmHg in control and did not differ in the three investigated pharmacological conditions, except that it was higher under double blockade than with metoprolol. Breathing frequency was 0.23±0.06 Hz in control and did not change in the three conditions. *At exercise*, in control condition, SAP was 138.5±17.5 and DAP was 60.9±7.5 mmHg. With respect to control, SAP was significantly lower under the three pharmacological conditions. No differences were observed for DAP. MAP was 86.8±9.9 mmHg in control and did not vary significantly among conditions. With respect to the corresponding values at rest, MAP during exercise was higher only in control. Breathing frequency was 0.42±0.07 Hz in control and did not change in the three other conditions.

HRV data are shown in Table 2. Examples of HRV spectra are shown in Figure 1. *At rest*, with respect to control, PTOT was not affected by metoprolol administration, but it was largely and significantly decreased under atropine and double blockade, due to drastically lower values of both LF and HF powers. No differences between atropine and double blockade were found. The same was the case at exercise, although the difference were much smaller than at rest, because, when moving from rest to exercise, PTOT was drastically reduced in control and under metoprolol. No differences for LF and HF powers between sympathetic blockade and control, or between atropine and double blockade, were observed.

At rest, the LF/HF ratio at rest was unaffected by drug treatment, the only significant difference being between atropine and double blockade. The same was the case for LFnu. No differences were observed concerning HFnu. *At exercise*, the LF/HF ratio did not differ under metoprolol or atropine with respect to control, but it

was lower under double blockade than in control and in the other pharmacological conditions. The same was the case for LFnu. Coherently, HFnu was higher in double blockade than in any other condition.

All data concerning spontaneous SAP and DAP variability are shown in Table 3. *At rest*, concerning SAP, no differences among conditions were observed for P_{TOT}. Concerning the LF power, no differences between sympathetic blockade and control were found, but it was lower under atropine and double blockade than in control and sympathetic blockade. The HF power in atropine and double blockade was lower than in control and under metoprolol, although for the latter the level of significance was not attained. *During exercise*, P_{TOT} was lower in all three investigated pharmacological conditions than in control, but no differences among conditions were observed for both the LF and the HF powers. In control and under atropine, the LF power was higher at exercise than at rest. The LF/HF ratio was unchanged in all conditions.

At rest, concerning DAP, no changes in P_{TOT} were found in any pharmacological condition with respect to control. The HF power did not vary among conditions, while the LF power was lower in atropine and double blockade than in control. The LF/HF ratio was lower in all conditions than in control. *During exercise*, there were no significant differences among conditions or with respect to the same condition at rest.

The BRS values at rest and exercise are shown in Figure 2. *At rest*, BRS was significantly lower under atropine and under double blockade than in control and under sympathetic blockade, which in turn did not differ between them. *During exercise*, BRS under atropine and double blockade was lower than in control and under sympathetic blockade. BRS was lower at exercise than at rest in all conditions except double blockade.

DISCUSSION

The analysis of spontaneous heart rate variability at rest showed that: 1. atropine administration drastically reduced PTOT, due to the fall of both LF and HF powers, with respect to control; 2. simultaneous double blockade with atropine and metoprolol provided the same results as atropine administration only; 3. metoprolol administration had no effects on heart rate variability.

When moving from rest to exercise, our results showed that: 1. no differences in PTOT, LF and HF appeared under atropine and under simultaneous atropine and metoprolol administration with respect to rest; 2. PTOT, and the LF and HF powers, were decreased by the same extent under metoprolol as in control. However, during exercise, PTOT, and the LF and HF powers were lower under atropine and double blockade than in control or with metoprolol.

These results are in line with the predictions made, and thus do not allow refutation of the vagal withdrawal hypothesis, but rather reinforce it. Although, taken separately, similar consistent results can be found in the previous literature (2, 6, 8, 10, 11, 15, 17, 21, 24, 29, 32, 33, 35, 43, 44), this is the first time that a complete picture of the role of the autonomic nervous system in determining heart rate variability in rest and exercise was obtained.

Heart rate variability

The significant increase in HR after atropine administration is in line with previous studies (9, 21, 22, 48, 50) and was opposed by the observation that, after metoprolol administration, despite a slight decrease, HR did not change significantly compared to control. These results were similar in size to those obtained in a previous study with the same drug (48). However, they are at variance with those of other studies, carried out in upright posture, showing a significant HR reduction at

rest with beta-blockade (11, 14, 15, 19). In supine posture, the predominance of vagal modulation of HR (20, 35) may explain the lack of HR changes with metoprolol.

Concerning HRV, metoprolol failed in changing PTOT, LF and HF at rest, indicating that a selective blockade of cardiac β-adrenergic receptors has no effects on spontaneous HR oscillations. This suggests that the sympathetic outflow to the heart may not be the main determinant of HRV, although the PTOT values under double blockade appear lower (just a tendency) than under atropine. These results for PTOT, although in agreement with those of some previous studies (15, 53), are in contrast with those by Cogliati et al. (11), who showed an increase in PTOT under atenolol, supporting the idea that the pattern was mostly due to an increase in the HF peak. This finding suggested stronger cardio-respiratory coupling under atenolol than in control. Comparable results were obtained by others (40) using propranolol.

Spontaneous HR oscillations were almost suppressed after atropine administration, as previously found (8, 15, 21, 29, 32, 33, 53), supporting the notion that parasympathetic outflow to the heart is the major determinant of HRV in resting humans. This was so also under simultaneous sympathetic and vagal blockade, indicating that suppression of the parasympathetic modulation of the heartbeat was the most important determinant of the present results. Breathing frequency did not change in the three conditions, being obviously higher at exercise than at rest. This implies that changes in HF power were not due to any change in breathing frequency.

Coherently, in the present study, passing from rest to exercise implied a large fall in LF and HF powers in control and under metoprolol. Conversely, under atropine and double blockade, in which a suppression of the vagal modulation of HR was obtained already at rest, no changes were found at exercise with respect to rest. These results demonstrate that the well-known fall of HRV, which is usually observed during exercise (37), is essentially a consequence of the withdrawal of the vagal

outflow to the heart occurring at exercise onset (12, 25), as hypothesized. As such, our results suggest that vagal withdrawal is incomplete at the investigated powers, because the LF and HF powers during exercise were still higher in control than with atropine or double blockade, the two conditions in which a full blockade of muscarinic receptors was attained. On the other hand, the fact that passing from rest to exercise generated comparable results with metoprolol as in control, is coherent with the reported decrease of the LF peak in humans (37, 39). These data are in contrast with the generally accepted notion that, during exercise, the degree of sympathetic activation increases (46, 54) and the modulation of the heartbeat by the sympathetic efferents becomes predominant (38, 45). This may mean that HRV in exercise does not reflect the degree of ongoing sympathetic activation.

When we look at the normalized variables at rest, none of the investigated drugs could change the LF/HF significantly with respect to control: this reflects the finding that the effects of drug administration on the LF and HF powers at rest were of the same size. In contrast, during exercise, there was a tendency toward a lower HF power than LF power. Yet this tendency, though not significant, was such as to provide, at exercise compared to rest, significantly lower HFnu values in control and under sympathetic blockade (only a tendency in A and in DB). Consequently, LF/HF ratio resulted higher at exercise than at rest, at least in these two cases.

In the context of the present hypothesis, this would suggest that the withdrawal of vagal tone at exercise onset might have had greater effects on the HF than on the LF component of HRV. Alternatively, the relative increase of the LF component of RR variability may suggest an increase of the cardiac sympathetic modulation. Nevertheless, LFnu in double blockade was significantly lower and HFnu significantly higher than in control, despite the lack of differences in the LF/HF ratio. This may be due to the non-autonomic effect of an increase in ventilation that is reflected on HRV through changes in venous return during exercise. A similar

condition can be observed in a neurodegenerative disease such as the pure autonomic failure. This condition is characterized by both a cardiac sympathetic and parasympathetic denervation leading to PTOT values mimicking high dosage atropine administration (16), in which a HF component of HRV, non-autonomic in origin, is present (39). These apparently contradictory results prevent us from arriving at clear-cut conclusions concerning the mechanisms at the basis of relative powers in this study.

Blood pressure variability

Arterial blood pressure at rest was unaffected by drug administration. The fact that atropine did not act on systemic blood pressure, in agreement with previous studies (15, 21), is coherent with the notion that there is no cholinergic innervation in most regional circulations. On the other hand, metoprolol is a selective blocker of β1-adrenergic receptors that are expressed specifically in the heart, not in arterioles, so that it is not expected to induce changes in blood pressure.

Coherently, SAP variability was much less affected by atropine and double blockade than HRV. According to Zhang et al. (61), who investigated spontaneous blood pressure variability under ganglionic blockade with Trimethaphan, the HF peak of blood pressure variability is mediated by mechanical effects due to the breathing cycle and cardiac filling: if this is so, one would not expect effects of any of the drugs used in this study on the HF power for blood pressure. In fact, the changes in HF power due to drug administration in the present study were much smaller than for HRV, although significant under atropine and double blockade. Zhang et al. (61) also attributed the LF power of blood pressure variability to either sympathetic activity or intrinsic vascular rhythmicity: if this is so, no changes in LF were to be found with atropine, metoprolol or double blockade: in fact, we found much smaller differences in LF power due to drug administration for blood pressure variability than for HRV.

Yet these changes were consensual with those of HF power, being significant under atropine and double blockade. These effects might have been an indirect consequence of the role that the autonomic nervous system may play in modulating the dynamic relationship between HRV and blood pressure variability (7, 61), with an involvement of its parasympathetic branch.

Most remarkable are the differences observed when passing from rest to exercise: the LF power for SAP increased in control, as expected (37, 39), and with atropine, but not with metoprolol and in double blockade. This indicates that the increase in LF power for SAP may be a consequence of increased sympathetic modulation during exercise. No effects were observed under any drug on the HF power: this means that the HF power of SAP is independent of the activity of the two branches of the ANS. The lack of exercise effects on HF power under drug stimulation explains why the P_{TOT} did not differ significantly at exercise with respect to rest under atropine.

DAP variability was unaffected by drug administration: this suggests that the exercise effect on the LF power of SAP, related to a selective blockade of $\beta 1$ adrenergic receptors, is mediated by a central (cardiac) rather than a peripheral (arteriolar muscle vasodilation) action of the sympathetic branch of the ANS.

Baroreflex sensitivity

At rest, BRS was drastically lower under atropine and double blockade than in control. This observation was consistent with what we observed for the LF peak of blood pressure variability: reduced under atropine and double blockade, unchanged under metoprolol, with respect to control. Coherently, when comparing rest with exercise in a given condition, BRS decreased in control and under metoprolol, but did not change under atropine and double blockade. These results on BRS appear in agreement with previous observations (1, 11, 56). Bringard et al. (4) postulated that

BRS is mainly modulated by the parasympathetic efferent branch on the ANS. These data support this hypothesis. Muscarinic receptors do not modulate smooth muscle tone in most arterioles, including those of skeletal muscles. Thus, the parasympathetic effect on arterial blood pressure variability indexes must be indirect. Based on the present results, we speculate that baroreflexes may participate in the modulation of the LF power of arterial blood pressure. The reduction of BRS observed during exercise (51) support the idea of alfa-index changes as previously reported (36). In the present study, the BRS reduction at exercise was observed only in control and with metoprolol, but not with atropine and double blockade. This finding reinforces the notion that withdrawal of vagal tone is responsible for the fall of BRS at exercise onset (4, 34). Coherently, no differences in BRS among the four investigated conditions were observed during exercise.

Study limitations

A limitation of this study may be suggested by the lack of differences between control and metoprolol, as this may also suggest that the β1-adrenergic blockade might have been incomplete. It is of note, however, that we used the same dose and followed the same procedure of metoprolol administration as in a previous study (14) in upright posture, which showed a significant resting HR decrease both in normoxia and in acute hypoxia at rest as at exercise. Moreover, we observe that the isoprenaline test provided unambiguous evidence of quasi-complete β1-adrenergic blockade.

Another possible limiting factor is related to the fact that HR rate differed remarkably among conditions. This may affect the HRV indexes in time domain *per se* (59), thus possibly undermining the relation to the action of the autonomic nervous system.

CONCLUSION

The results of this study support the tested hypothesis that vagal suppression is responsible of the disappearance of the spontaneous HRV during exercise. The observed effects on arterial blood pressure variability are indirectly related to the action of the administered drugs, supporting the notion that blood pressure and HRV are only partially-associated phenomena, possibly controlled by different physiological mechanisms

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Table 1: Mean steady state values for the cardiovascular variables monitored during rest (R) and exercise (E) in the four experimental conditions: Control, Atropine, Metoprolol, and Double Blockade.

Measured variables		Control	Metoprolol	Atropine	Double Blockade
HR (min^{-1})	R	62.67±8.47	59.58±7.11$^{\#\S}$	111.17±17.75*§	93.71±5.48*
	E	**105.04±12.39**	**93.53±8.17**$^{\#}$	**135.04±20.56***	103.19±8.06$^{\#}$
RR (ms)	R	985.3 ± 185.7	1017.7 ± 104.4$^{\#}$	548.6 ± 79.5 *§	642.1 ± 38.2 *
	E	**577.9 ± 66.2**	**645.3 ± 51.3**$^{\#}$	455.9 ± 89.3*	584.2 ± 41.1$^{\#}$
SAP (mmHg)	R	111.97±9.52	109.75±13.89	112.96±11.83	119.48±14.29
	E	**138.51±17.53**	113.58±15.21*	108.73±15.94*	107.70±14.76*
DAP (mmHg)	R	54.95±9.64	48.96±10.81§	60.95±9.10	66.16±8.43
	E	60.94±7.48	53.35±13.55	54.21±7.72	**54.34±6.92**
MAP (mmHg)	R	73.95±8.59	69.22±10.42§	78.28±7.76	83.93±7.78
	E	**86.79±9.88**	73.42±13.53	72.58±10.03	72.13±9.41
BRS (ms $mmHg^{-1}$)	R	25.74 ± 11.28	27.42 ± 8.51$^{\#}$	2.17 ± 1.06 *§	3.00 ± 0.92 *
	E	**2.59 ± 1.76**	**3.17 ± 0.62** $^{\#}$	**0.85 ± 0.31***	2.13 ± 0.44
BF (Hz)	R	0.23 ± 0.06	0.21 ± 0.06	0.29 ± 0.04	0.23 ± 0.07
	E	**0.42 ± 0.07**	**0.39 ± 0.04**	**0.40 ± 0.04**	**0.41 ± 0.07**

Values are means ± SD. HR: heart rate; RR: R-R interval; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MAP: mean arterial pressure; BRS: spontaneous baroreflex sensitivity. BF: breathing frequency.

N=7; 2-way ANOVA for repeated measurements; $p<0.05$; *: significantly different from Control. #: significantly different from Atropine. §: significantly different from Double Blockade. In bold: Exercise significantly different from Rest.

Table 2: Mean and standard deviations of all parameters calculated by means of heart rate variability in the four investigated conditions: Control, Atropine, Metoprolol, and Double Blockade.

Heart Rate variability		Control	Metoprolol	Atropine	Double blockade
ABSOLUTE					
P_{TOT} (ms^2Hz^{-1})	**R**	6351.4 ± 4476.4	7883.2 ± 5965.9	22.5 ± 13.8*$^{\bullet}$	12.9 ± 4.9*$^{\bullet}$
	E	**185.4 ± 77.1**	**93.6 ± 30.9***	10.1 ± 3.3*$^{\bullet}$	14.8 ± 4.7*$^{\bullet}$
LF (ms^2Hz^{-1})	**R**	1717.5 ± 1290.6	2711.9 ± 2061.8	1.5 ± 1.2*$^{\bullet}$	1.1 ± 0.5*$^{\bullet}$
	E	**40.6 ± 29.3**	**41.3 ± 29.3**	1.7 ± 1.4*$^{\bullet}$	1.6 ± 1.5*$^{\bullet}$
HF (ms^2Hz^{-1})	**R**	1441.0 ± 1296.1	2552.3 ± 2245.0	0.9 ± 0.5*$^{\bullet}$	2.6 ± 0.8*$^{\bullet}$
	E	**10.8 ± 7.8**	**11.2 ± 9.2**	0.3 ± 0.13*	3.1 ± 1.6*$^{\bullet}$
RELATIVE					
LF/HF	**R**	1.4 ± 1.0	1.4 ± 0.8	1.8 ± 0.9	0.5 ± 0.3$^{\#}$
	E	**4.1 ± 2.0**	**4.0 ± 2.0**	4.0 ± 2.6	0.3 ± 0.1*$^{\bullet\#}$
LFnu (%)	**R**	46.8 ± 19.3	46.1 ± 14.7	57.7 ± 28.2	25.9 ± 13.8$^{\#}$
	E	69.6 ± 16.5	65.3 ± 21.2	61.6 ± 22.7	12.7 ± 8.0*$^{\bullet\#}$
HFnu (%)	**R**	51.1 ± 18.3	51.1 ± 15.4	38.3 ± 26.9	62.6 ±15.6
	E	**15.5 ± 8.7**	**17.4 ± 5.0**	22.6 ± 14.4	45.5 ± 23.5*$^{\bullet\#}$

Values are means ± SD. P_{TOT}: total power. LF: low frequency power. HF: high frequency power. LF/HF: low–to–high frequency ratio; LFnu, relative low frequency power; HFnu, relative high frequency power. R: Rest. E: Exercise.

N=7; 2-way ANOVA for repeated measurements; $p<0.05$; *: significantly different from Control. ●: significantly different from Metoprolol. #: significantly different from Atropine. In bold: Exercise significantly different from Rest

Table 3: Parameters resulting from the analysis of spontaneous variability of systolic and diastolic arterial pressures in the four investigated conditions: Control, Atropine, Metoprolol, and Double Blockade.

SAP variability		**Control**	**Metoprolol**	**Atropine**	**Double Blockade**
P_{TOT} (ms2Hz^{-1})	**R**	25.70 ± 11.52	26.91 ± 15.50	16.99 ± 17.77	15.63 ± 8.19
	E	**70.83 ± 41.42**	29.07 ± 12.24*	28.09 ± 6.77*	17.46 ± 7.00*
LF (ms2Hz^{-1})	**R**	7.03 ± 3.60	4.96 ± 1.90	1.55 ± 0.64*$^{\bullet}$	2.09 ± 1.38*$^{\bullet}$
	E	**18.68 ± 17.97**	5.51 ± 1.52	**10.93 ± 6.15***$^{\bullet}$	5.80 ± 2.90*$^{\#}$
HF (ms2Hz^{-1})	**R**	4.04 ± 3.21	2.57 ± 1.79	1.20 ± 0.45*	1.09 ± 0.67*
	E	5.49 ± 4.20	5.48 ± 3.96	3.29 ± 1.80	2.48 ± 0.93
LF/HF	**R**	2.27 ± 1.07	2.36 ± 1.09	1.46 ± 0.70	2.32 ± 1.54
	E	2.61 ± 1.39	1.87 ± 1.32	3.46 ± 2.39	2.17 ± 0.67
DAP variability					
P_{TOT} (ms2Hz^{-1})	**R**	9.65 ± 6.06	9.01 ± 3.47	4.64 ± 3.03	5.10 ± 2.32
	E	7.63 ± 2.56	5.52 ± 2.56	3.92 ± 1.00	4.90 ± 3.40
LF (ms2Hz^{-1})	**R**	3.54 ± 2.57	2.56 ± 1.17	0.77 ± 0.52*	0.97 ± 0.67*
	E	2.70 ± 1.80	1.97 ± 0.86	1.63 ± 0.48	1.11 ± 0.31
HF (ms2Hz^{-1})	**R**	2.22 ± 2.77	1.88 ± 2.10	0.40 ± 0.20	0.46 ± 0.52
	E	1.66 ± 1.25	1.13 ± 0.75	0.89 ± 0.52	0.92 ± 0.50
LF/HF	**R**	3.65 ± 1.26	2.40 ± 1.21*	2.03 ± 1.26*	3.03 ± 1.48*
	E	3.00 ± 2.53	1.69 ± 0.98	2.05 ± 0.73	1.53 ± 0.63

Values are means ± SD. P_{TOT}: total power. LF: low frequency power. HF: high frequency power. LF/HF: low–to–high frequency ratio. R: Rest. E: Exercise.

N=7; 2-way ANOVA for repeated measurements; $p<0.05$; *: significantly different from Control. ●: significantly different from Metoprolol; #: significantly different from Atropine. In bold: Exercise significantly different from Rest

Figure 1: Heart Rate Variability (HRV) spectrum resulting from the experiments which the shown HRV segments belong to *left column*: Rest; *right column*: Exercise; *first row*: Control; *second row*: Atropine; *third row*: Metoprolol; *fourth row*: Double blockade. N=7; X axis: frequency (Hz). Y axis: RR power (ms2/Hz). Note: differences in Y scales. C: Control. A: Atropine. M: Metoprolol DB: Double blockade.

Figure 2: Mean values ± SD of BRS in each investigated condition (control / atropine / metoprolol / double blockade) at rest and during exercise. BRS: Spontaneous baroreflex sensitivity. N=7; 2-way ANOVA for repeated measurements; $p<0.05$: *: significantly different from CTRL. #: significantly different from DB. §: significantly different from ATR. $: significantly different from the same condition at rest.

Figure 1

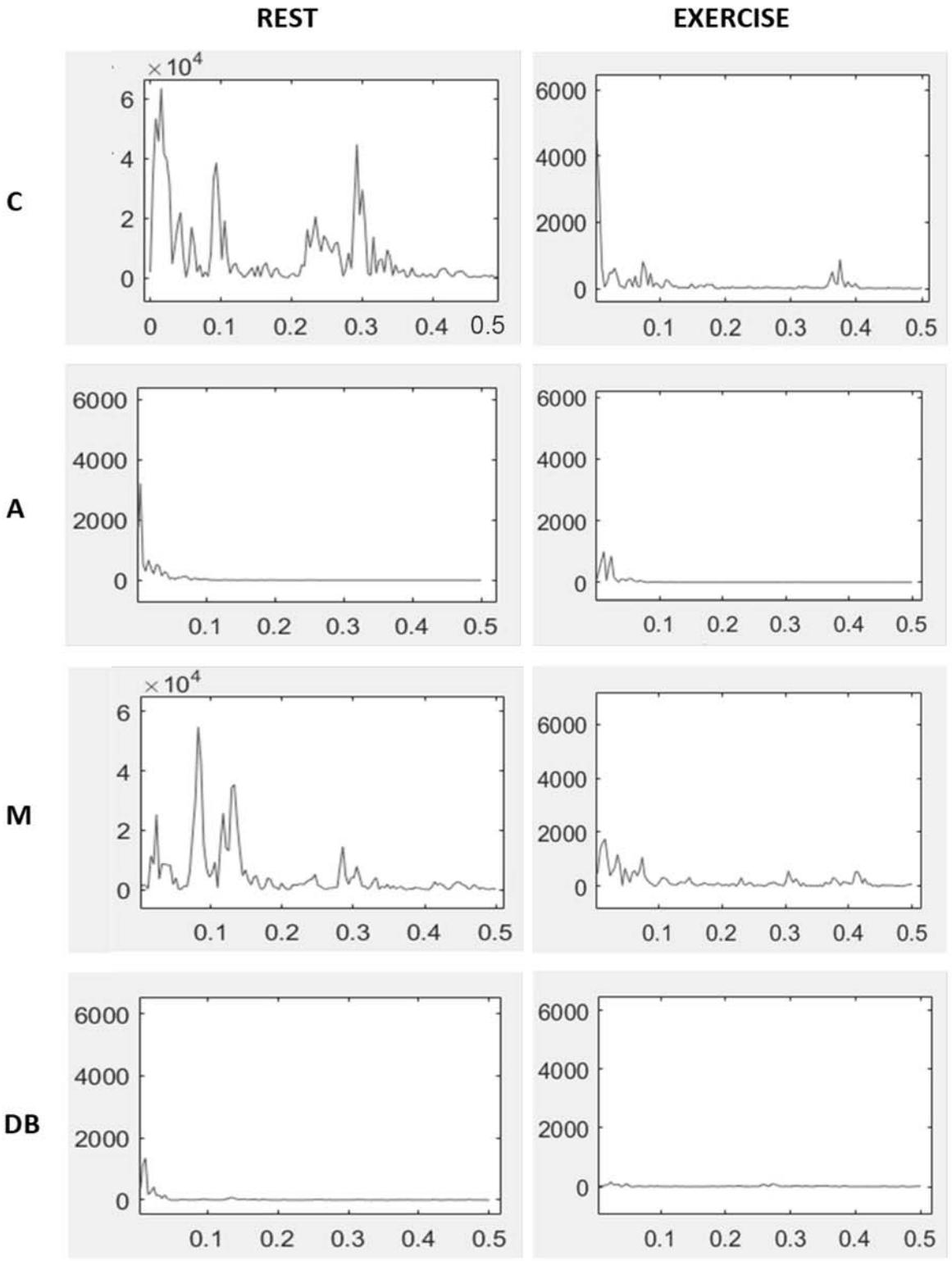

Figure 2